AAG AGC ACG TTT CTT CTT TTT (forward) (SEQ ID NO: 9). The two purified fragments were then assembled together in a second PCR reaction with the external primers 818A and 818D (30 cycles: 1 sec at 94°C, 1 sec at 50°C, 10 sec at 72°C). The amplified product from this final reaction was purified, digested with EcoRI and HindIII and ligated into the corresponding site of *pMEX.SakSTAR*. For each construction, the sequence of the variant was confirmed by sequencing the entire *Sak*STAR coding region.

IN THE CLAIMS:

Please cancel claims 1-12 and add the following new claims 13-21:

13. (New) A staphylokinase derivative having essentially the amino acid sequence as depicted in Figure 3 in which one or more encircled or boxed amino acids have been replaced by another amino acid thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase and further incorporating one or more polyethylene glycol groups.

14. (New) The staphylokinase derivative of claim 13 in which one polyethylene glycol group is coupled in position 102 thereon.

C9

15. (New) The staphylokinase derivative of claim 13 in which the staphylokinase specific activity of the derivative is at least 50 percent that of wild type staphylokinase.

16. (New) Staphylokinase derivative SakSTAR(K35X,G36X,E65X,K74X, E80X,D82X,K102X,E108X,K109X,K121X,K130X,K135X,K136X,+137X) having the amino acid sequence as depicted in Figure 1 in which the amino acids Lys in position 35, Gly

Back to PALM |ASSIGNMENT | OASIS| Home page

in position 36, Glu in position 65, Lys in position 74, Glu in position 80, Asp in position 82, Lys in position 102, Glu in position 108, Lys in position 109, Lys in position 121, Lys in position 130, Lys in position 135 and/or Lys in position 136 have been replaced with other amino acids and/or in which one amino acid has been added at the COOH-terminus, thus altering the immunogenicity after administration in patients, and further incorporating at least one polyethylene glycol group.

17. (New) Staphylokinase derivatives listed in Tables 1, 2, 3, 4, 5, 6, 7 and 8, having the amino acid sequence depicted in figure 3 in which the boxed or encircled amino acids have been replaced by other amino acids thus reducing the absorption of SakSTAR specific antibodies from plasma of patients treated with staphylokinase, without reducing the specific activity, the derivative further incorporating at least one polyethylene glycol group.

C9

18. (New) Method for producing the staphylokinase derivatives as claimed in claim 13, comprising the steps of:

a. preparing a DNA fragment comprising at least the part of the coding sequence of staphylokinase that provides for its biological activity;

b. performing in vitro site-directed mutagenesis on the DNA fragment to replace one or more codons for wild-type amino acids by a codon for another amino acid;

c. cloning the mutated DNA fragment in a suitable vector;

d. transforming or transfecting a suitable host cell with the vector; and

e. culturing the host cell under conditions suitable for expressing the DNA fragment.

19. (New) Method as claimed in claim 18, wherein the DNA fragment is a 453 bp *Eco*RI-*Hin*dIII fragment of the plasmid *pMEX602sakB*, the *in vitro* site-directed mutagenesis is performed and the mutated DNA fragment is expressed in *E. coli*.

20. (New) Pharmaceutical composition comprising at least one of the staphylokinase derivatives as claimed in claim 13 together with a suitable excipient.

C9

21. (New) Pharmaceutical composition as claimed in claim 20 for treating arterial thrombosis.